toxines of the affection from which the organism is suffering. In other words, normal temperature is preserved by a mutual see-saw action of these centres—the thermogenetic and the thermolytic.

We recognise fully that, for a more perfect understanding of death temperatures, it is necessary for the observers to examine the patients for themselves, and not to trust to records, however many or accurate they may be, so that they can note in each case the changes in the skin, the circulation, the respirations, etc., concurring with the variations of the bodily temperature. Nevertheless, we venture to put forward our investigation and views, not as physiologists, but as clinical observers, with the hope of pointing out new lines of research, by which may be increased the knowledge of the regulation of animal heat.

"A Note on the Action of Radium on Micro-organisms." By Alan B. Green, M.A., M.D. (Cantab.). Communicated by Sir Michael Foster, K.C.B., F.R.S. Received April 11,—Read May 5, 1904.

[Plate 11.]

The radium salt used in these experiments was 1 centigramme of radium bromide, bought of Messrs. Buchler and Co., of Brunswick, in June, 1903. It was contained in a vulcanite and brass capsule, fronted with thin talc. The radium was enclosed immediately behind the talc, and the circular area over which it was spread was about 3 mm. in diameter. The radium emanations which were applied to micro-organisms were such as passed through the talc, *i.e.*, the β and γ rays.

Dr. E. F. Bashford, to whose kindness I am greatly indebted for the use of the radium, has informed me that Sir William Ramsay tested the preparation for the intensity of its combined β and γ rays, the latter being a practically negligible quantity. The results showed that, on comparison with samples of radium bromide giving a virtually pure spectrum of radium, these rays were practically 100 per cent. This radium salt was, in fact, a pure preparation of radium bromide.

Dr. Bashford also informs me that this radium bromide caused pigment to disappear after 18 days from a mole with 15 minutes' exposure, the talc being in contact with the surface of the mole. Five minutes such exposure produced a marked skin reaction, while 20 minutes' exposure caused a reaction proceeding almost to ulceration.

I found that the radium was itself luminous, and that it could

illuminate a screen of zinc sulphide through a sheet of lead over 1 cm. thick. It discharged a gold leaf electroscope, highly charged with + or − electricity, at a distance of over 6 feet. It caused a brown colouration of glass or talc when applied at a distance of 1 mm. for 12—24 hours.

The experiments which have been made are of two kinds. In the first set investigation was made of the germicidal action of radium emanations, and in the second, endeavours were made to ascertain whether micro-organisms exposed to the emanations became thereby themselves radio-active.

I.—*The Germicidal Action of Radium Emanations.*

The following have been subjected to the emanations of radium:—

(*a*) Calf vaccine, together with its contained extraneous bacteria, which in these experiments consisted of *S. pyogenes aureus*, *S. pyogenes albus*, *S. cereus flavus*, *S. cereus albus*. Both freshly collected and stored calf-vaccine pulp were exposed to radium emanations in the following way:—A layer of pulp not exceeding 0·5 mm. in thickness was spread in the centre of the depression of a hollow-ground glass slide, around the circumference of which depression a metal ring had been cemented. The capsule containing the radium bromide was placed upon the metal ring in such a way that the salt was brought within 1—2 mm. of the lymph pulp, nothing separating them but the talc of the capsule and the intervening air. The pulp was thus used in the thinnest practicable layer, in order that the emanations might act as uniformly as possible on all the component parts.

The vitality of the vaccine and of its extraneous bacteria was tested before, and at varying intervals of time after the exposure to radium; the former by inoculations on calves, the latter by cultivations on nutrient media. For each such test a small portion of vaccine was removed from the preparation and was mixed with enough sterile water to form a semi-fluid emulsion. A loopful of emulsion was used to inoculate a liquefied tube of nutrient agar-agar, and a plate was established in the usual way. The remainder of the emulsion was used for inoculating a calf.

(*b*) The following species of micro-organisms have also been separately subjected to the action of radium:—*S. pyogenes aureus*, *S. pyogenes albus*, *S. cereus flavus*, *S. cereus albus*, *Streptococcus pyogenes*, *B. prodigiosus*, *B. proteus vulgaris*, *B. pyocyaneus*, *B. typhosus*, *B. coli communis*, *B. mallei*, *B. pestis*, *B. tuberculosis*, the bacillus of Malta fever, *Spirillum choleræ Asiaticæ*, and sporing cultures of *B. mesentericus vulgatus*, *B. mesentericus ruber*, *B. subtilis*, *B. anthracis*, *B. tetani*, Gärtner's bacillus, the bacillus of malignant œdema, and the bacillus of Rauschbrand.

In the case of these micro-organisms, growth was removed from solid media and was exposed to the radium emanations as a film in the depression of a hollow-ground slide, the radium being applied as in the case of vaccine. The vitality of these micro-organisms was tested both before and after exposure to radium by cultivations on media by a similar method to that used in the case of vaccine.

In some instances metal rings were placed round colonies of bacteria *in situ* on the surface of nutrient medium, and exposure of bacteria under these conditions made by placing the capsule containing the radium on the rings.

In the case of each experiment with vaccine and with micro-organisms in pure culture, a control was carried out by making a similar preparation and subjecting it to similar conditions as the experimental preparation with the exception of exposure to radium; it was also subjected to tests for vitality similar to those used for the experimental preparation, at corresponding intervals of time. All experiments and their controls were made at room temperature.

The exposure of micro-organisms in liquid media to radium was found unsatisfactory owing to the presence of material of a complex nature between the radium and the micro-organisms, and owing also to the constant variation in the distance between the radium and the micro-organisms suspended in the liquid.

It was found from the foregoing experiments and their controls that a marked germicidal action was exerted on the specific and extraneous micro-organisms of vaccine and on the other above-mentioned micro-organisms as a result of their exposure to the radium at a distance of 1—2 mm. for varying lengths of time.

The following is a summary of the results of these experiments and their controls:—

Results of Experiments with Vaccine.

The specific germ in no case survived a longer exposure to radium than 22 hours, at the end of which time it had completely lost its ability to cause vesiculation or any visible irritation at the site of inoculation on a calf. In seventeen out of a total of twenty-five experiments its potency was destroyed after 10 hours' exposure to radium and in four cases after 2 hours. The controls remained fully potent after the experimental vaccines had been rendered inert.

The extraneous micro-organisms of these vaccines, as has been previously mentioned, consisted of *S. pyogenes aureus*, *S. pyogenes albus*, *S. cereus flavus*, *S. cereus albus*. In each experiment these bacteria were destroyed after exposure to radium in rather less time than was the potency of the specific germ. In no case did they survive a longer exposure to radium than 15 hours.

The extraneous micro-organisms of the control vaccines were alive

after those of the experimental vaccines had been killed. The following experiment may be related to explain in greater detail this action of radium.

Experiment.

On November 12, 1903, vaccine pulp was exposed to radium at 10 A.M. Portions of this pulp were removed from the influence of the radium at the end of 2, 6, and 10 hours' exposure. Liquefied nutrient agar-agar tubes were inoculated with one platinum loopful of each portion immediately after its removal from the radium, and plates were established in the usual way. The remainder of each portion was inoculated on a calf on the following day, November 13.

Control.

Plates were similarly established from the control at 10 A.M. and 8 P.M. on November 12, and the remainder of the vaccine was used for inoculating the calf on the following day, November 13.

On November 18, the experimental portion of vaccine exposed to radium for 2 hours had caused good vesiculation on the calf; the portion exposed for 6 hours caused very poor vesiculation, and the remaining portion exposed for 10 hours caused no trace of vesiculation.

The number of extraneous bacteria originally present in the vaccine were 1200 per platinum loopful of emulsion (the mixture of this emulsion has been previously described). In the portion of vaccine exposed to radium for 2 hours the number left alive was 1050 per platinum loopful; in the portion exposed for 6 hours there were fifty bacteria, and in the portion exposed for 10 hours there was no evidence of living bacteria at all.

On November 18 the control portion of the vaccine had caused good vesiculation; and the agar-agar plate poured at 8 P.M. on November 12 contained practically the same number of colonies of extraneous bacteria as were present in the plate poured from the same vaccine at 10 A.M. on the same date.

Results of Experiment with Non-sporebearing Bacteria.

All the non-sporebearing bacteria previously mentioned were killed after exposure to radium for 2—14 hours. A description in detail of the results of some of these experiments may be of use in illustrating this germicidal action.

One of a Series of Experiments with S. pyogenes aureus.

Before exposure to radium, plate cultivations showed 84,000 bacteria present per platinum loopful of emulsion. After exposure to radium for 6 hours, this number had decreased to 31,000; at the end of 10 hours' exposure to 260, and at the end of 14 hours' exposure no bacteria were left alive.

At the end of 14 hours the control preparation showed bacteria alive in practically undiminished numbers.

One of a Series of Experiments with B. coli communis.

Before exposure to radium, 75,000 bacteria were present per platinum loopful of emulsion. After 3 hours' exposure this number was reduced to 3000, and after 6 hours' exposure all the bacteria were killed.

The control at this time showed practically the same number of bacteria as were present originally.

One of a Series of Experiments with Spirillum choleræ Asiaticæ.

Before exposure to radium, 47,000 bacteria were present in a platinum loopful of emulsion. After 3 hours' exposure to radium, 2100 were left alive per platinum loopful, while no bacteria survived an exposure of 6 hours.

The control preparation showed at this time practically no decrease in the number of bacteria originally present.

Results of Experiments with Bacteria containing Spores.

Bacteria containing spores were by far the most resistant to the germicidal action of radium of any micro-organisms used in these experiments, for they were not killed by less than 72 hours' exposure. This corresponds with the time given by R. Pfeiffer and E. Friedberger* as necessary for the killing of spores by the emanations of the radium used by them.

The following are examples of experiments with these micro-organisms :—

One of a Series of Experiments with B. mesentericus vulgatus (*Sporing*).

Immediately before exposure to radium the preparation showed 170,000 micro-organisms per platinum loopful of emulsion. After 48 hours' exposure this number had decreased to 260, and at the end of 72 hours all micro-organisms per platinum loopful had been killed.

The control preparation showed practically no decrease in the number of micro-organisms at the end of 72 hours.

One of a Series of Experiments with B. anthracis (*Sporing*).

There were originally present 11,000 micro-organisms per platinum loopful. After 48 hours' exposure to radium, 120 only were left alive, and at the end of 72 hours all micro-organisms per platinum loopful were killed.

The control showed micro-organisms present in undiminished numbers at the end of 72 hours.

One of a Series of Experiments with B. tetani (*Sporing*).

In the experiments with tetanus spores the actual numbers of micro-organisms were not investigated, but only the presence or absence of living germs by means of cultivations in the depth of sugar agar.

After 48 hours' exposure to radium, the presence of living micro-organisms was still evidenced, but after 72 hours' exposure no growth followed the inoculation of a sugar agar tube.

Thus in these experiments the non-sporebearing bacteria exhibited the least resistance to the germicidal action of radium emanations, withstanding exposure for 2—15 hours only.

The resistance of the specific germ of vaccine was slightly in excess

* 'Berl. Klin. Woch.,' July 13, 1903.

of this; while by far the greatest resistance was shown by spores, these not being killed by less than 72 hours' exposure.

Experiments have also been made from which the following points have been noted.

1. As the distance between the radium and the micro-organisms subjected to its emanations was increased, the germicidal action which was marked at the nearest distance became less evident and finally ceased to be exerted.

In these experiments *Staphylococcus pyogenes aureus* was used, a separate strain being used for each series of experiments. Portions of growth were subjected to the radium emanations for the same time and under the same conditions, except that the distance between the radium and the bacteria was varied. After 30 hours' exposure it was found that—

At 1 mm. bacteria were killed.

At 1 cm. bacteria were usually lessened in numbers, but all were not killed.

At 10 cm. no definite germicidal action was apparent.

The following is an example:—

Experiment. *Staphylococcus pyogenes aureus* exposed to radium emanations for 30 hours at different distances.

Series.	Number of bacteria originally present per platinum loopful of emulsion.	Number of bacteria present per platinum loopful of emulsion after 30 hours' exposure to radium.		
		At 1 mm.	At 1 cm.	At 10 cm.
1 (*a*)	1060	0	..	..
1 (*b*)	1700	..	160	..
1 (*c*)	1200	..	..	987

2. As extra thicknesses of mica or glass were interposed between the radium and the micro-organisms exposed to their influence, the time of germicidal action was delayed. Finely woven copper gauze also caused slight delay of germicidal action. A sheet of lead 0·1 mm. thick, placed between the radium and the micro-organisms, caused weakening of germicidal action, and as extra thicknesses of lead were interposed and the β-rays were cut off, germicidal action became less and less evident.

II.—*Induced Radio-Activity of Bacteria.*

It has been found that after exposure at a distance of 1 mm. to the radium emanations for 24—120 hours, micro-organisms themselves may

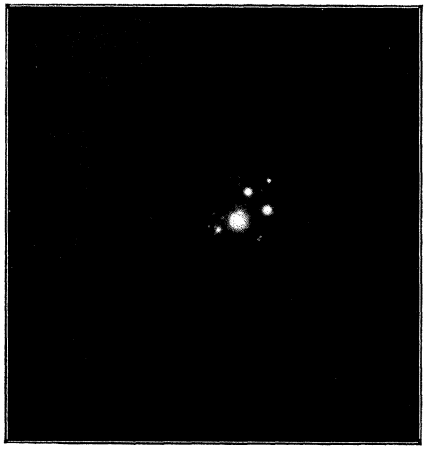

FIG. 1.—Photograph of a mass of *B. mesentericus vulgatus* (sporing), made radio-active by exposure to radium bromide for 72 hours and killed by the exposure.

FIG. 2.—Photograph, taken through a double layer of lead-foil, of a mass of *B. mesentericus vulgatus* (sporing), made radio-active by exposure to radium bromide for 72 hours and killed by the exposure.

show signs of radio-activity. It has not yet been ascertained whether living micro-organisms can exhibit induced radio-activity, but micro-organisms which have been killed by exposure to the radium emanations can do so.

In these experiments no radio-activity has been found in bacteria not exposed to the action of radium.

Induced radio-activity of micro-organisms has been shown in the following manner :—A small mass of bacteria, removed from the surface of nutrient medium, after subjection to the radium emanations at a distance of 1 mm., for, as a rule, 72 hours, was removed from the depression of the hollow-ground glass slide in which it had been exposed to the emanations, and was placed between two thin sheets of glass, generally coverslips, which were not themselves radio-active. These sheets of glass, with the small mass of bacteria pressed between them, were next, in a dark room, brought into contact with the film of an Ilford "special rapid" photographic plate. A cotton-wool pad was placed on the glass sheets to keep them in position, and the whole was wrapped up in a light-proof package. Twenty-four hours later the photographic plate was developed and a photograph was obtained of the bacterial mass. An image has been developed after only 1 hour's exposure of a sensitised plate to the radio-active bacteria, and in some instances after a fortnight's exposure.

Faint images have been thus produced on sensitised plates by *S. p. aureus* and *albus* which had been subjected to radium emanations, but, so far, the best photographs have been obtained from bacterial masses containing a number of spores, after their subjection to the emanations for 24—120 hours (Plate 11, fig. 1).

Radio-active micro-organisms have continued to give off photo-actinic emanations after 3 months have elapsed since their exposure to radium.

Photographs of masses of micro-organisms, possessing induced radio-activity, have been obtained through a double layer of lead foil (Plate 11, fig. 2). A sheet of lead 3 mm. in thickness interposed between a radio-active bacterial mass and the sensitised plate has prevented the passage of photo-actinic emanations from the glass to the plate. These photographs would seem, therefore, to be caused by β-rays emitted by the micro-organisms.

My best thanks are due to Mr. Power, Medical Officer of the Local Government Board, for the facilities he has afforded me in the research, and for the kind and valuable advice he has given me concerning it.

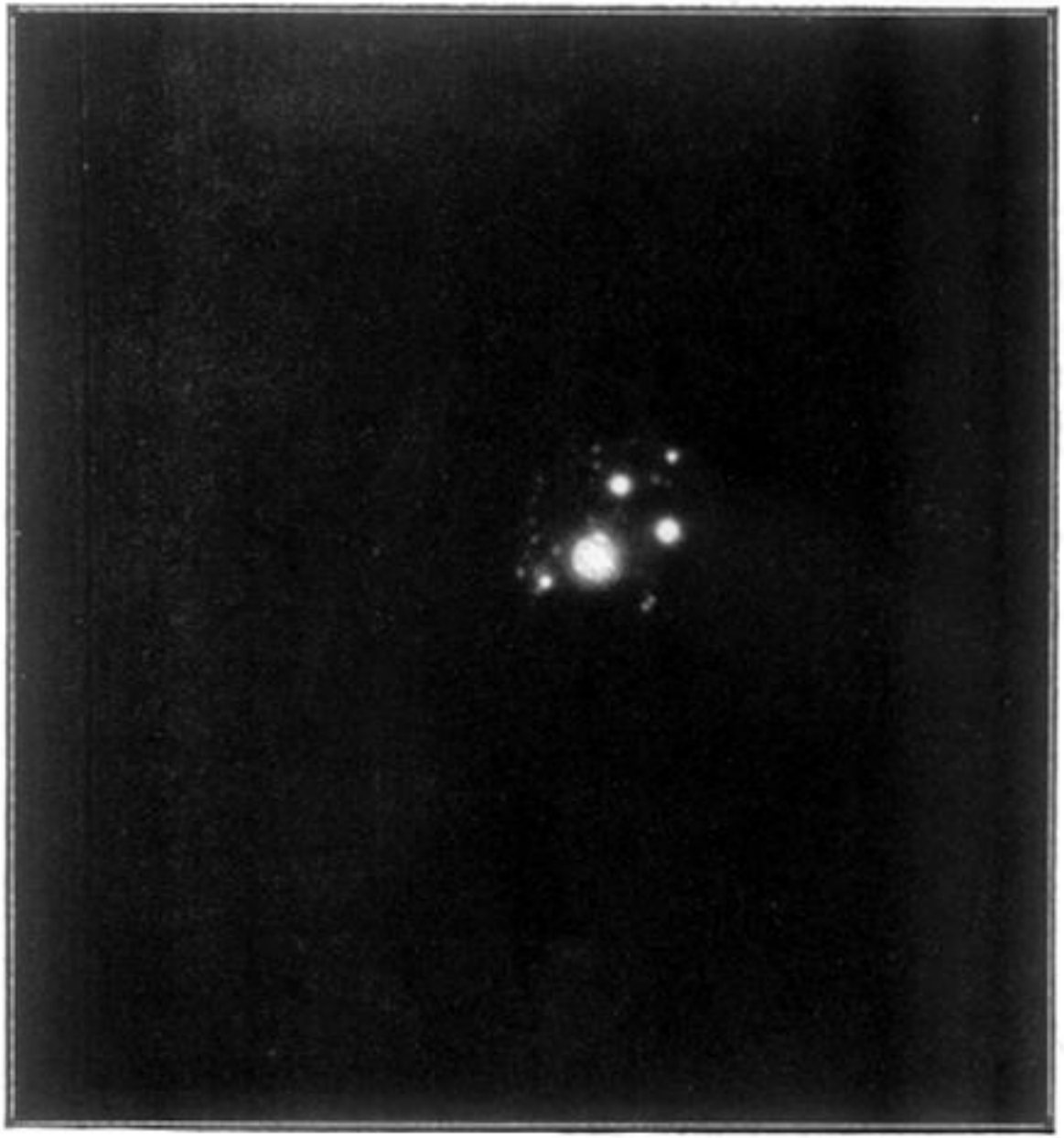

FIG. 1.—Photograph of a mass of *B. mesentericus vulgatus* (sporing), made radio-active by exposure to radium bromide for 72 hours and killed by the exposure.

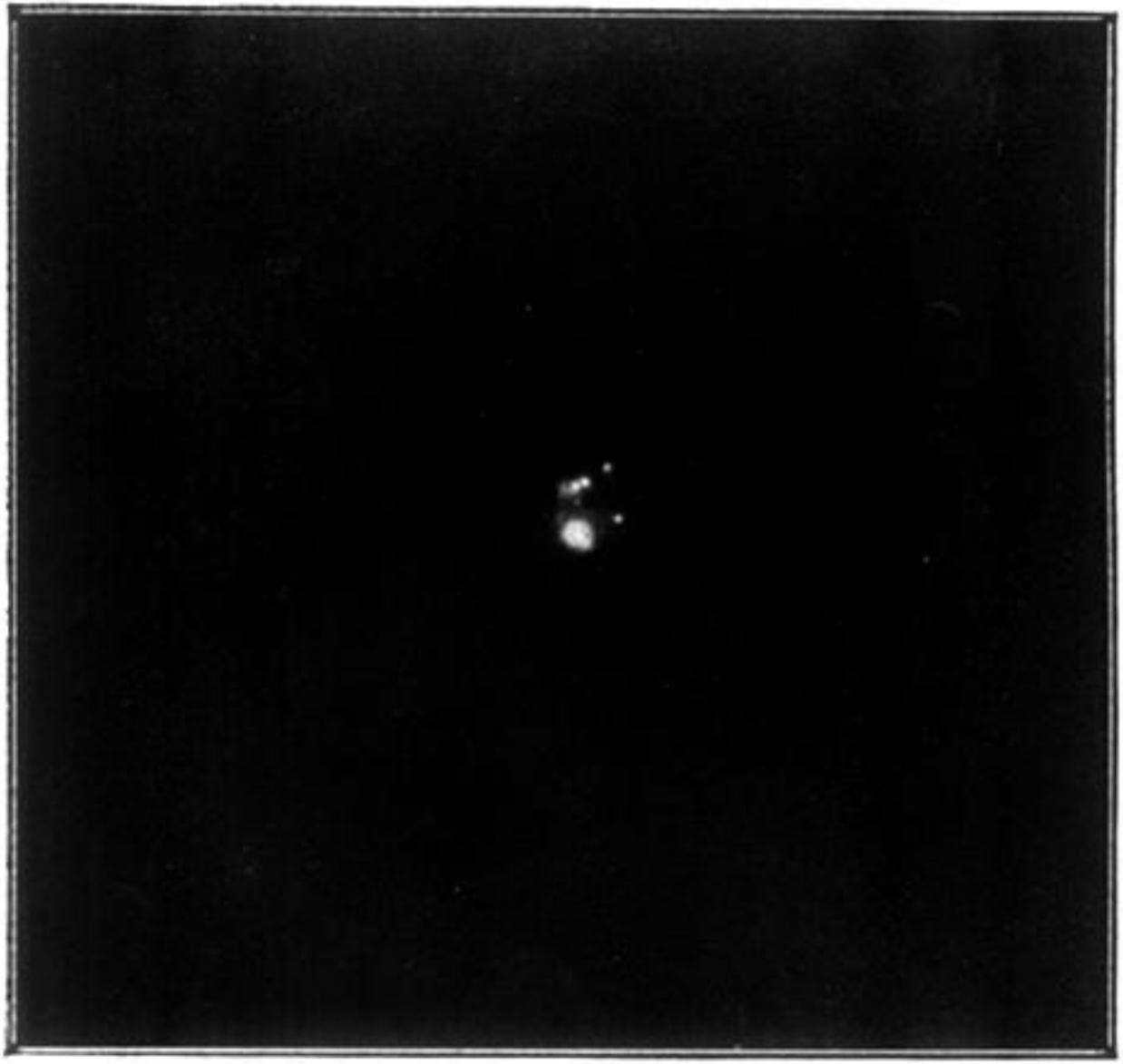

FIG. 2.—Photograph, taken through a double layer of lead-foil, of a mass of *B. mesentericus vulgatus* (sporing), made radio-active by exposure to radium bromide for 72 hours and killed by the exposure.